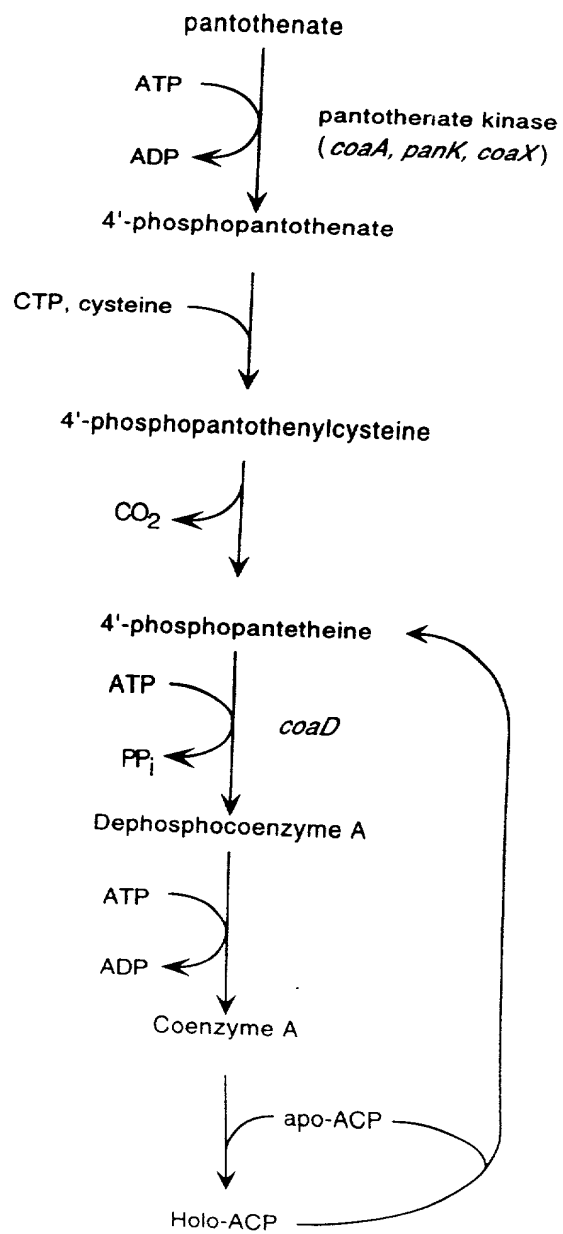
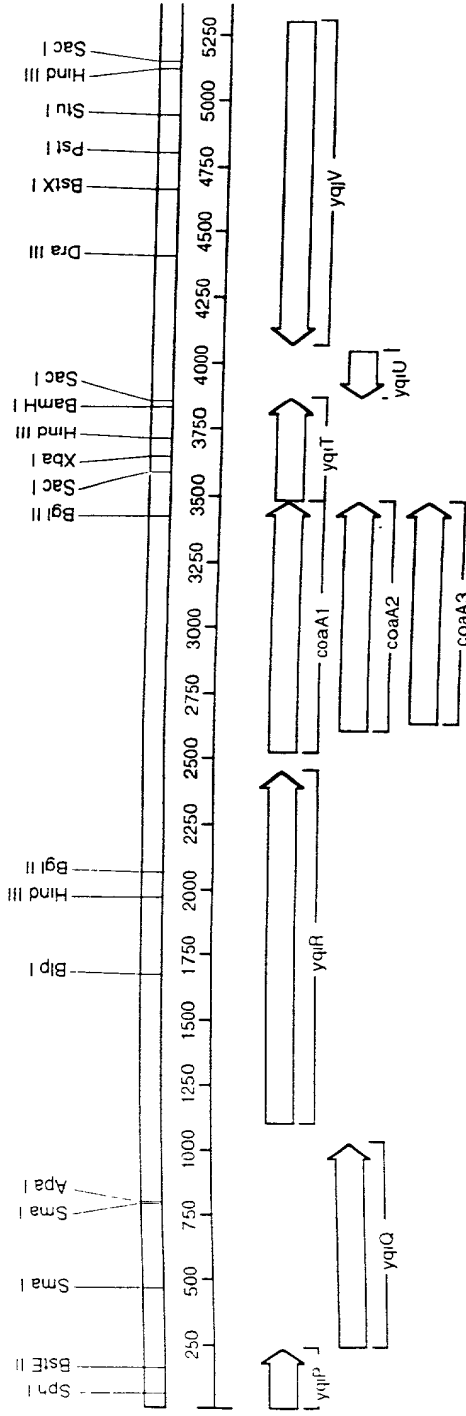


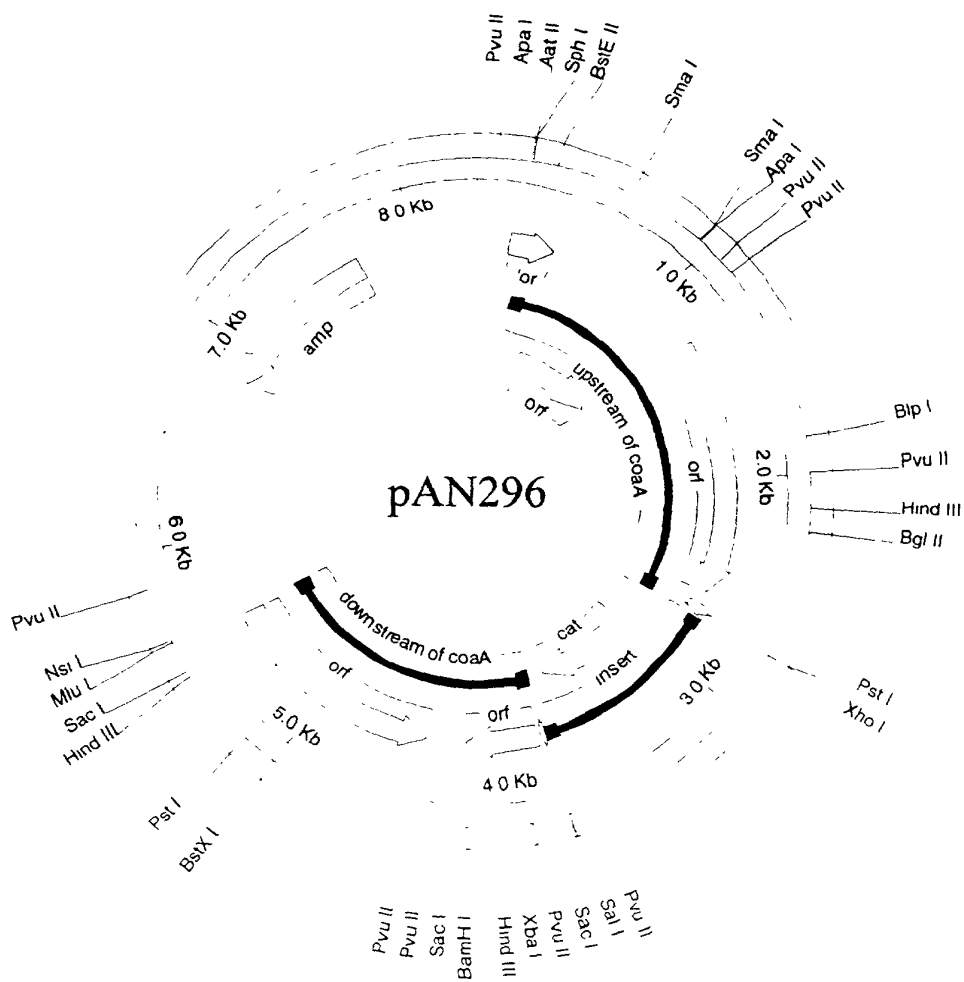
Figure 26.7

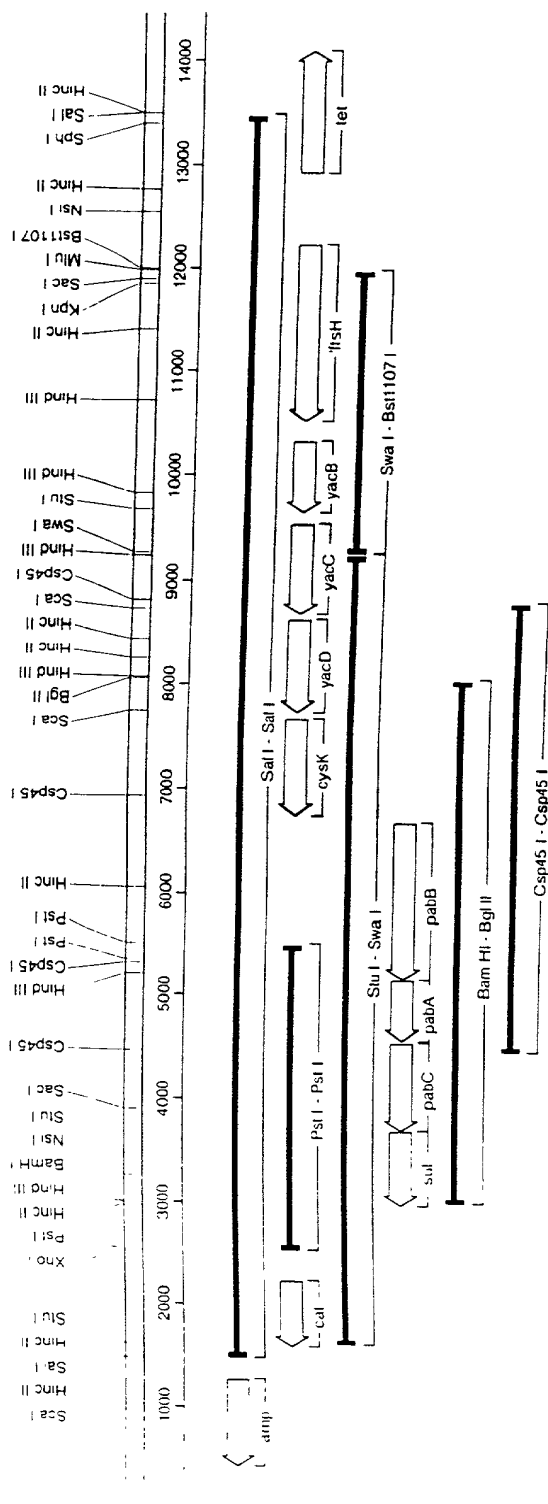


2  
 Figure 8 Structure of the *B. subtilis* chromosome in the region of the *coaA* gene. The scale is in base pairs and the significant open reading frames are shown by the open arrows.

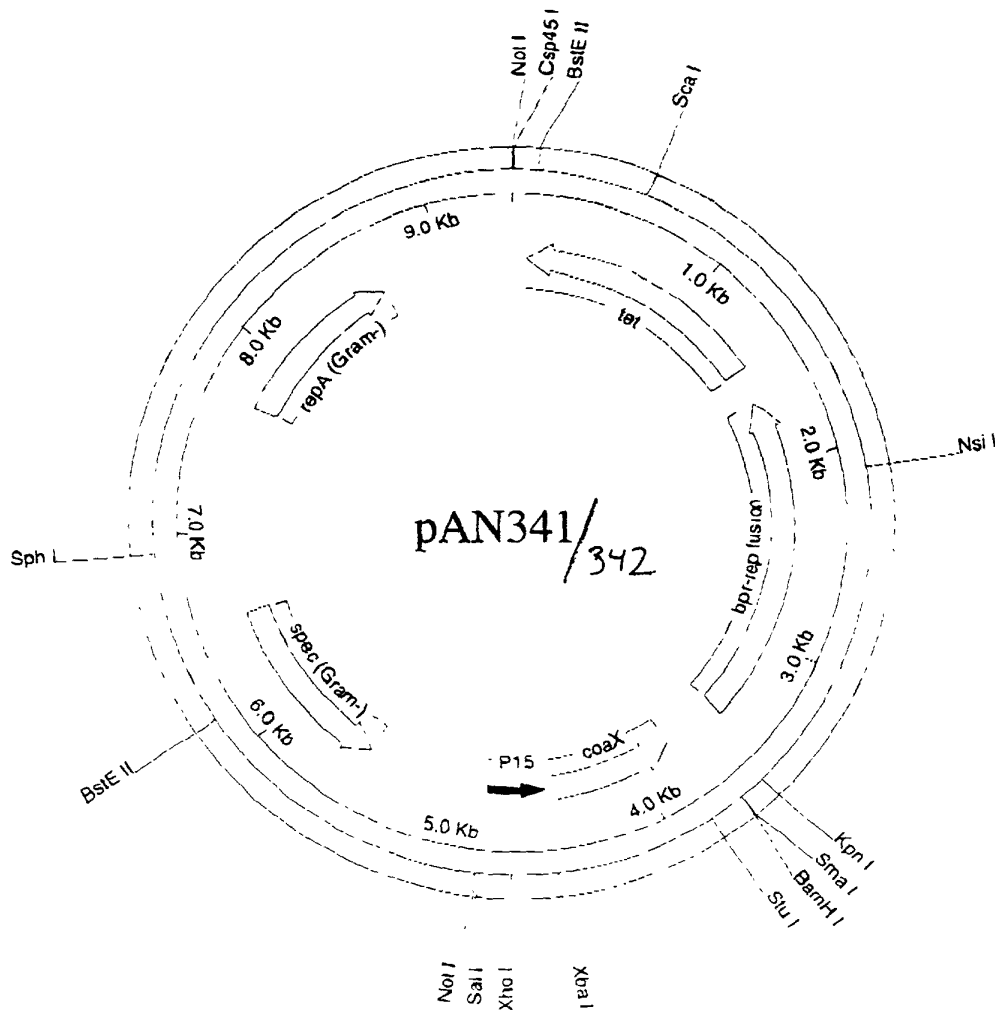


13  
 Figure 1 Structure of pAN296, designed to delete most of the *B. subtilis* *coaA* gene and substitute a chloramphenicol resistance gene.





5  
**Figure 27** Structure of pAN341 and pAN342, two independent PCR-derived clones of *yacB* (renamed *coaX*).



CLUSTAL W (1.7) Multiple Sequence Alignments

6A  
FIG.28A

CLUSTAL W (1.7) Multiple Sequence Alignments

Sequence type explicitly set to Protein

|                                  |        |                                       |        |
|----------------------------------|--------|---------------------------------------|--------|
| Seq 1: B.subtilis Coax SEQNO.2   | 258 aa | Seq 8: sp O51477 B.burgdorferi        | 262 aa |
| Seq 2: dbj BAA21476.1 D.vulgaris | 212 aa | Seq 9: sp P74045 Synecocystis         | 257 aa |
| Seq 3: gb AAD35964.1 T.maritima  | 246 aa | Seq 10: sp O25533 H.pylori            | 223 aa |
| Seq 4: pir T36391 S.coelicolor   | 265 aa | Seq 11: sp O67753 A.aeolicus          | 229 aa |
| Seq 5: sp Q45338 B.pertussis     | 267 aa | Seq 12: sp Q9RX54 D.radiodurans       | 262 aa |
| Seq 6: sp O06282 M.tuberculosis  | 272 aa | Seq 13: WIT RCA03301 C.acetobutylicum | 250 aa |
| Seq 7: sp O83446 T.pallidum      | 273 aa | Seq 14: WIT RRC02473 R.capsulatus     | 258 aa |

|                               |                                                    |
|-------------------------------|----------------------------------------------------|
| B.subtilis Coax SEQIDNO.2     | -----MLLVIDVGNNTNVLGVYHDG-----KLEYHWRIE            |
| WIT RCA03301 C.acetobutylicum | NKRAAFMLLLFLRSVLKVLVLDVGNNTNVLGIYNDT-----KLTAEWRLS |
| pir T36391 S.coelicolor       | -----MLLTIDVGNTHVLGLFDGE-----DIVEHWRIS             |
| sp O06282 M.tuberculosis      | -----MLLAIDVNTHTVVGLLSGMKEHAKVVQQWRIR              |
| WIT RRC02473 R.capsulatus     | -----MLLCIDCGNTNTVFSVWDGT-----DFAATWRIA            |
| dbj BAA21476.1 D.vulgaris     | -----MTQHELLFDIGNTNVKIGIAVET-----AVLTSYVLP         |
| sp Q9RX54 D.radiodurans       | -----MPAFPLAVDIGNTTVLGLADASG-----ALHTWRIR          |
| gb AAD35964.1 T.maritima      | -----MYLLVDVGNTHSVFSITEDG-----KTFRRWRIS            |
| sp O83446 T.pallidum          | -----MLLIDVGNSHVVFQI QGENGRCVCVRELFRIA             |
| sp O51477 B.burgdorferi       | -----MNKPLLSLI IDIGNTSIAFALFKDN-----QVNLFIKMK      |
| sp O67753 A.aeolicus          | -----MRFLTVDVGNSSVDIALWEGK-----KVK                 |
| sp P74045 Synecocystis        | -----METSKPGCGALDNDKQKPLGLMIGN-----SRLHWAYC        |
| sp O25533 H.pylori            | -----MPARQSFTDLKN--LVLCDIGN-----TR                 |
| sp Q45338 B.pertussis         | -----MIILDSGNSRLKVGWFDPDAP--QAAREPAPV              |

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6B

FIG. 23B

B. subtilis|Coax|SEQIDNO\_2  
WIT|RCA03301|C.acetobutylicum  
pir|T36391|S.coelicolor  
sp|O06282|M.tuberculosis  
WIT|RR02473|R.capsulatus  
dbj|BAA21476.1|D.vulgaris  
sp|Q9RX54|D.radiodurans  
gb|AAD35964.1|T.maritima  
sp|O83446|T.pallidum  
sp|O51477|B.burgdorferi  
sp|O67753|A.aeolicus  
sp|P74045|Synechocystis  
sp|O25533|H.pylori  
sp|Q45338|B.pertussis

B. subtilis|Coax|SEQIDNO\_2  
WIT|RCA03301|C.acetobutylicum  
pir|T36391|S.coelicolor  
sp|O06282|M.tuberculosis  
WIT|RR02473|R.capsulatus  
dbj|BAA21476.1|D.vulgaris  
sp|Q9RX54|D.radiodurans  
gb|AAD35964.1|T.maritima  
sp|O83446|T.pallidum  
sp|O51477|B.burgdorferi  
sp|O67753|A.aeolicus  
sp|P74045|Synechocystis  
sp|O25533|H.pylori  
sp|Q45338|B.pertussis

TSRHKTEDEFGMTLSLFDHS---GLMFEQIDGIISSVWPPIMPALER  
TDVLSADEVGIQVMNLFQQD---KLDPTLVEGVIISSVVPNIMYSLEH  
TDSRTADELAVLLQGLMGHPLLGLDELGDIGDIAICATVPSVLHELRE  
TESEVTADELALTIDGLIG-----EDSERLTGTAAALSTVPSVLHEVRI  
TDHRTADSYFVWLNTLMQLK-----GLQGRISIAIISSTAPRVFNLRV  
TDPGOTTDSIGLRLLLEVLRHAG---LGPADVGACVASSVPGVNPILIRR  
TNREMLPDLALQLHGLFTLA-----GAP-IPRAAVLSSVAPVGENYAL  
TGVFQTEDELSHLHPLLG-----DAMREIKGIGVASVPTQNTVIER  
PDARKTQDEYSLLIHALCERAG---VGRASLRDAFTSSVVPVLTKTIAID  
TNLMRLYDEVYSFFEENFDEN-----VN---K-VFISSVVPILNETFRN  
DFLKLSHDEEFLKEEFPKLK-----ALGISVKQSFSEKVRG  
SGNAPLQTVWTDYNPKSAQLP-----VLLGKVPLMLASVWPE  
IHFAQNTQLFSSAKEDLKR-----LGIQKEIFYISVNEE  
AFDNLDLDALGRWLATLPRP-----Q-----RALGVNVAGLARGEAIA

MCTKYFHIPEQIVG-PG-MKTGLNLIKYNPKVEVGADRIVNAVAATHLYG-  
MIRKYFKINPLVVG-PG-IKTGINIKYNPKVEVGADRIVNAVAHEIYK-  
VTRRYGDPVAVLVEFG-VKTGVPIILTDHPKVEVGADRIINAVAAVELYG-  
MLDQYWPSPVPHLIEPG-VRTGIPELVNPKVEVGADRIVNCILAAVDRFR-  
LCNRYFDCRPYVVGKPG-CELPVAPRVPDGTTVGPDRLVNTVAGYDRHG-  
ACERYL--YRKLIFAPGDIAIPLDNRYERPAEVEGADRLVAAVAAARLYP-  
ALKRHFMDAPFAVSAEN--LPDVTVELDTPGSGVADRLCNLFGAEKYLG-  
FSQKYFHIPTWVKAEN---GCVKMNKNPSEVGADRVANVAVFVKEYG-  
AVAQISGVQPVVGPWAYEHLVPRIPPEVRAEIGTDLVANAVAAVHFR-  
VIFSFFKIKPLFIGFDLNDLTFTNPKYSDKFLGSDVFAVLVAAIENYS-  
KIPKIK-----FLKEN---FPIQVDYKTPETLGTDRVALAYSAKKFYG-  
QTEVWRVYQPKILITLKN---LPLVNLVY---SFGIDRALAGLGTGLTYG-  
NEKALLNCYPNAKNIAG--FFHLETDYVG---LGIDRQMACLA---VN--  
ATLRAGGCDIRWLRAQP-LAMGLRNGYRNPDLQLGADRWACMVGVLARQPS

\* \*

60

B. subtilis|Coax|SEQIDNO\_8  
WIT|RCA03301|C.acetobutylicum  
pir|T36391|S.coelicolor  
sp|006282|M.tuberculosis  
WIT|RCA02473|R.capsulatus  
dbj|BAA21476.1|D.vulgaris  
sp|Q9RX54|D.radiodurans  
gb|AAD35964.1|T.maritima  
sp|083446|T.pallidum  
sp|051477|B.burgdorferi  
sp|067753|A.aeolicus  
sp|P74045|Synechocystis  
sp|025533|H.pylori  
sp|Q45338|B.pertussis

WIT|subtilis|Coax|SEQIDNO:4  
WIT|RCA03301|C.acetobutylicum  
pir|T36391|S.coelicolor  
sp|006282|M.tuberculosis  
WIT|RRC02473|R.capsulatus  
dbj|BA21476.1|D.vulgaris  
sp|Q9RX54|D.radiodurans  
gb|AAD35964.1|T.maritima  
sp|083446|T.pallidum  
sp|051477|B.burgdorferi  
sp|067753|A.aeolicus  
sp|P74045|Synechocystis  
sp|025533|H.pylori  
sp|Q45338|B.pertussis

NP--LIIVDFGTAFTTCYIDENKQYMGAAIPGITIITSTALYSRAAKLPR  
RS--LI IIDFGTAFTTCVAVRENGDYLGGAICPGIKVSSALFEKAALPR  
GP--AIIVDFGTAFTTDAVSARGEYIGVVIAPGIELSVBALGVKAQRLR  
KA--AIIVDFGSSICVDVWSAKFEGALAIAPGVOSDPAARASQAULR  
GD--LIIVDFGTAFTTDDVAPDGA YIGVVIAPGVNLSEALHMAAALPH  
GPRSLVSDFGTAFTTDCVGE--GAYLGLICPGVLSSAGALSRTAKLPR  
GLDVAIVDVGFTFTNFDDVGRGRFELGGLIATGAQVSADALFARAALPR  
KN--GIIIDMGTAFTTVDLVN--GSYEGAILPGFTFMVHSLFRTAKLPL  
SA--CVVDDGTAFTTAVDGTGLIQGVAIAPGLTAVQSHTGTAQPL  
FEN--VLVDLGTACTIFAVSRQDGLGINSGLPNFNLSDNAYLIKE  
KN--VWVTSAGPALVIDLVLE--GKFGGFTITGLGCKKLILSPDAEGIPK  
FP--CLVVDGTAFTTGTGDDKKLVGAILPGLQLQIATLGDRLAALPK  
NG---VVVDAGSAITIDLKE--GKHLGGCILPGLAQYTHAYKSAKTLEQ  
VHPPLLVASFGTATTLDTIGPDNVFPGLLILPGPAMMRGALAYGTAHLPL

IETPRDN--IIGKNTVSAMQSIGLFGYVGQVEGIVKRMKWQAKQDLK-  
 VELIKPAY--AICKNTISSIQSIGVIVRYLQVKYLFEXLKENLPQGRRT  
 IETAPRS--VIGKNTVEAMQAGVYFGAGQDGVNRMARELAD--P  
 VELAPRS--VIGKNTVEAMQAGVYFGAGQDGVNRMARELAD--P  
 VDVTKPQG--VIGNTVACIQSGVYNGYIGLVEGIVGRIEMRDRP--  
 ILSVEEEDS--PVIKSGTSTSLNHGFIFGFAAMTEGVLA--  
 ITLPQPET--AIKGNTHVALQSGLVGYAMTEGDLRLIRAEIPGE--  
 VEKPADF--VVGKDETEENIRLGVNGSVYALEGIGRIKEYVGDL-  
 VPLALPDS--VLGRDTHIAQAGVVRGTLFVIRAMIAQOCELGR--  
 FPISTPN--LLEKRTSGVNSGLFYQYKVLIEGVYRDIKOMYKK--  
 FPEVEEI--FLGRSTRECVLGA YRESTEFTKSTLKLWRKVRFRK--  
 LEMQDTLPEDRALDTPS AIFSGVYVYIGALQSYLQDWQKLFPCA--  
 PFKALDSL--EVLPKSTRDAVNYGMVLSV IACTIOHLAK--NQK--  
 ADGLVADY-----PIDTHQIAISRAAQAGAIVRQWLGRAGORYGQAP--  
 \*  
 \*



60  
FIG. 230

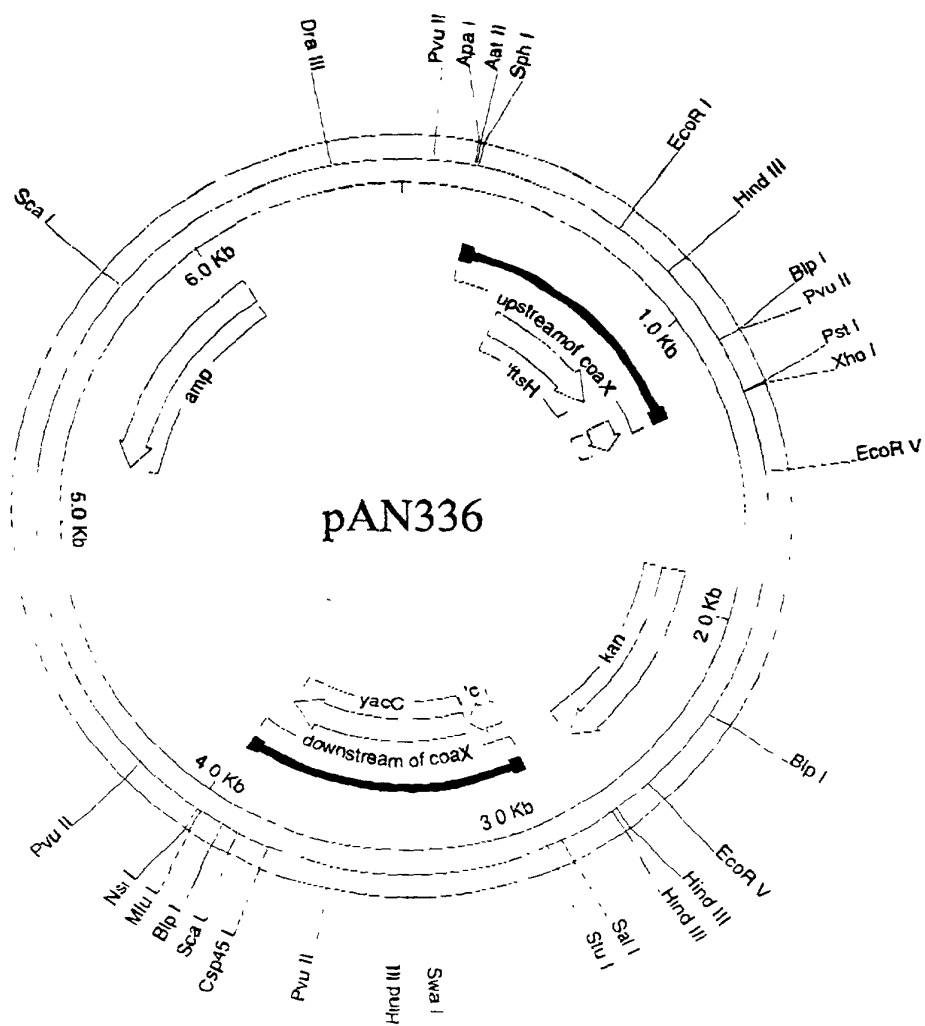
*B. subtilis* | Coax | SEQIDNO 2  
WIT | RCA03301 | C. acetobutylicum  
pir | T36391 | S. coelicolor  
sp | O06282 | M. tuberculosis  
WIT | RRC02473 | R. capsulatus  
dbj | BAA21476.1 | D. vulgaris  
sp | Q9RX54 | D. radiodurans  
gb | AAD35964.1 | T. maritima  
sp | O83446 | T. pallidum  
sp | O51477 | B. burgdorferi  
sp | O67753 | A. aeolicus  
sp | P74045 | Synechocystis  
sp | O25533 | H. pylori  
sp | Q45338 | B. pertussis

*B. subtilis* | Coax | SEQIDNO 2  
WIT | RCA03301 | C. acetobutylicum  
pir | T36391 | S. coelicolor  
sp | O06282 | M. tuberculosis  
WIT | RRC02473 | R. capsulatus  
dbj | BAA21476.1 | D. vulgaris  
sp | Q9RX54 | D. radiodurans  
gb | AAD35964.1 | T. maritima  
sp | O83446 | T. pallidum  
sp | O51477 | B. burgdorferi  
sp | O67753 | A. aeolicus  
sp | P74045 | Synechocystis  
sp | O25533 | H. pylori  
sp | Q45338 | B. pertussis

-----VIATGG-----LAPIANES-----DCIDIVDPFLLKGLLELI  
RTSLVLTATGG-----LAKLIN-----  
DDVTVIATGG-----LAPMVLGES-----SVIDEHEPWLTLMGLRLV  
HDVAIVATGH-----TAPLLLPGL-----HTVDHYDQHLTLQGLRLV  
-----MKVIATGG-----LASLFDLGF-----DLFDKVEDDLTMHGLRLI  
-----  
-----AVAVATGG-----FSRTVQIGC-----QEIDYVDETLTLRGLVEL  
-----VLTGG-----QSKIVK-DM-----IKHEIFDEDLTIKGVYHF  
-----CAAVITGG-----LSRLFS-SE-----VDFPPIDAQTLTSLGLAHI  
-----FNLIIITGG-----NADLILSLI-----EIEFIFNIHLTVEGVRIL  
-----FKVVIITGG-----EGKYFS-----KFGIYDPLLVHVRGMRNL  
-----AMVITGG-----DGKILHGFLKEHSPNLSVAWDDNLIIFLGMAAI  
-----IYLCGG-----DAKLSAFL-----PHSVCKERLVFDGMEIA  
-----EIYVAGGGWPEVQEAERLLAVTGAAFGATPQPTYLDSPVLDGLAAL

YERNRVGSV-----  
-----  
YERNVSRM-----  
FERNLEVQGRGLKTAR-----  
FDYNGKGLGA-----  
-----  
WASRSEVR-----  
CFGD-----  
ARLVPTSLPPATVSGSSGN  
GNSIDFRFVN-----  
LYLYHRI-----  
HHGDRPIC-----  
LKKAGILECK-----  
AAQGAPTA-----

**Figure 2** Structure of pAN336, a plasmid designed to delete *B. subtilis* *coaX* from the chromosome and replace it with a kanamycin resistance gene.



# Construction of pOTP72

Fig 8

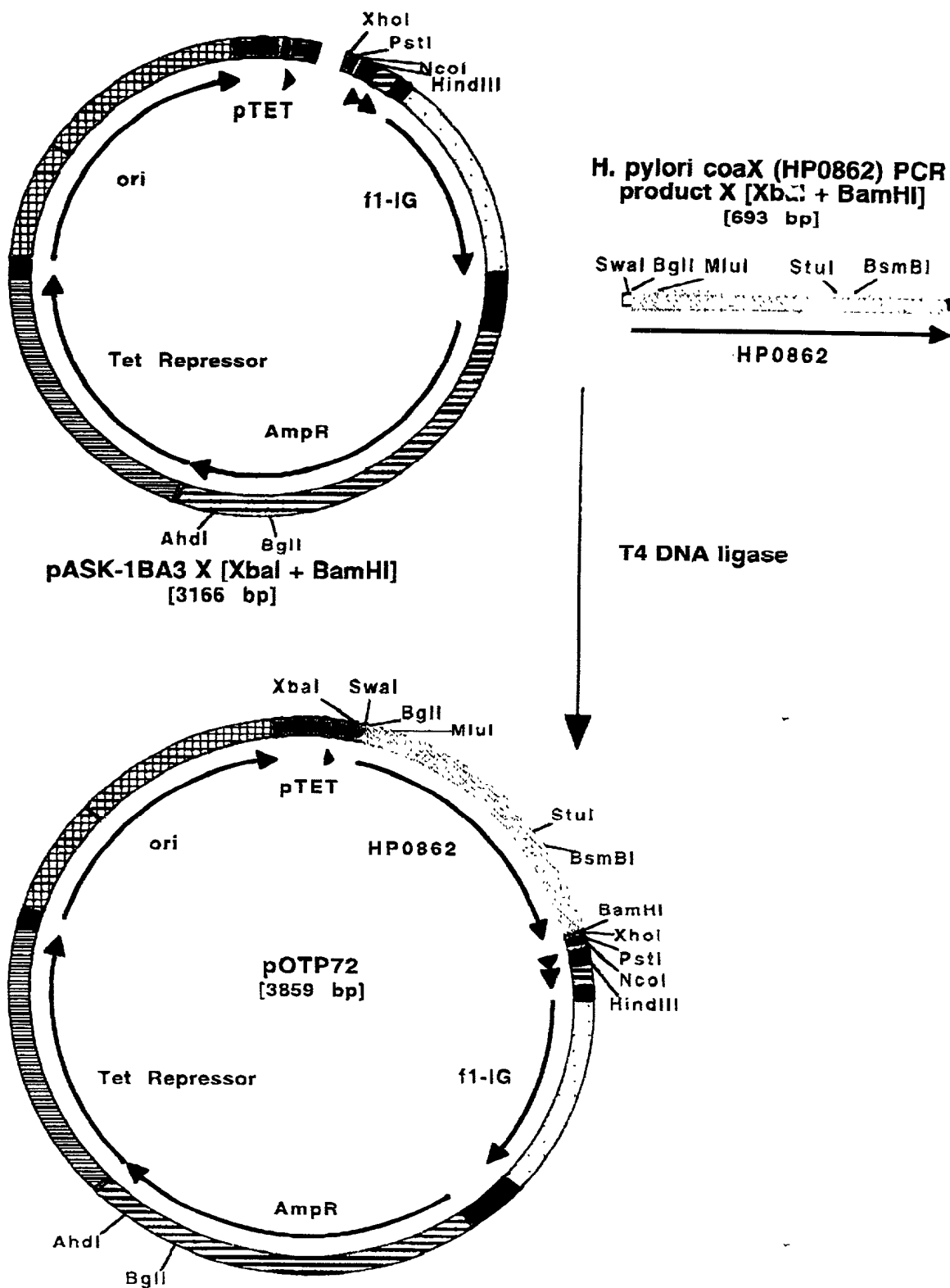


Fig 9

# Construction of pOTP73

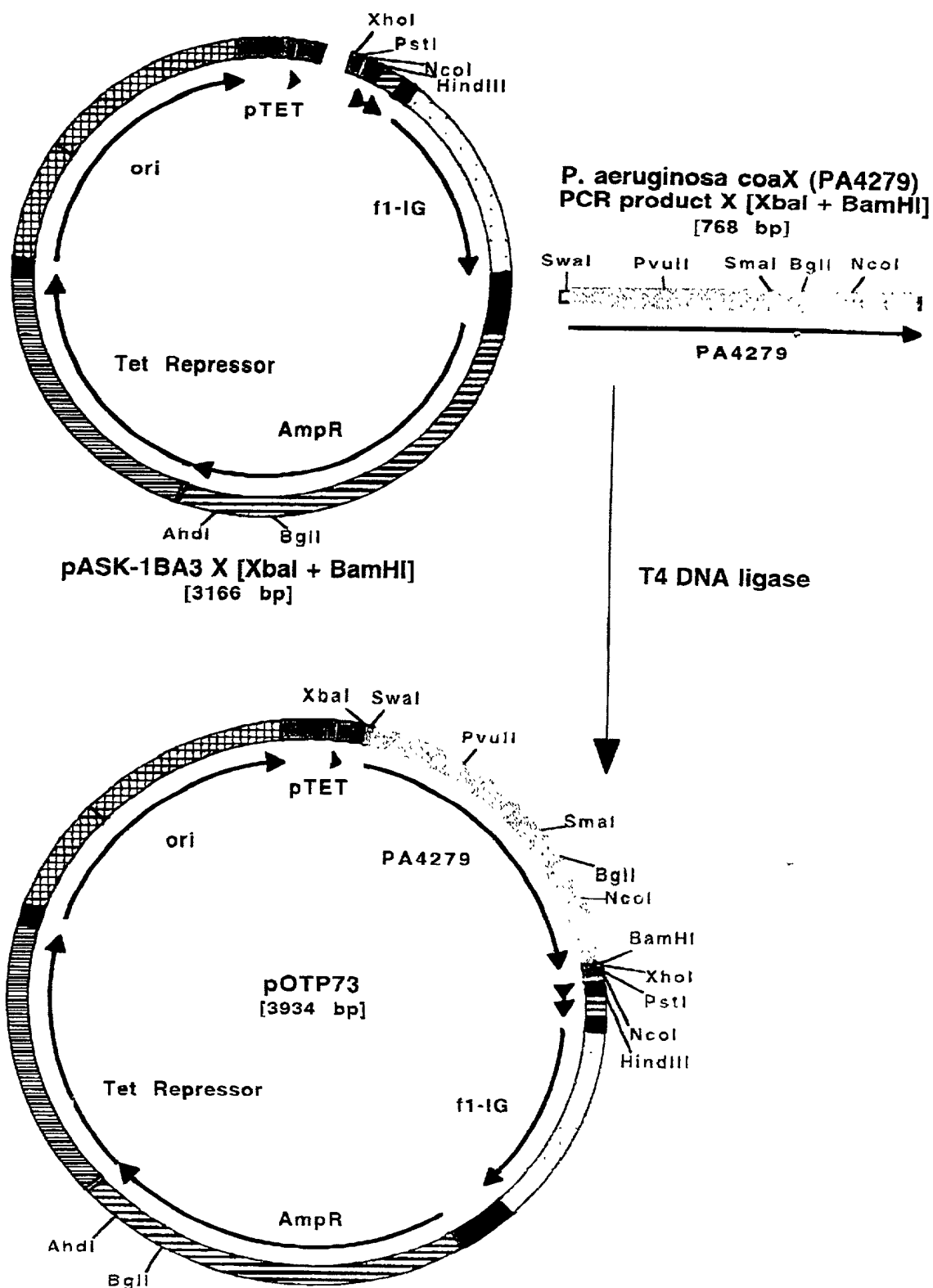


Fig 10

# Construction of pOTP71

